Staphylococcus aureus (*S. aureus*) Virulence Factors

By

Jaden Skelly

Senior Honors Thesis

Department of Biology

University of North Carolina at Chapel Hill

May 5th, 2021

Approved:

_____

Dr. Brian Conlon, Thesis Advisor

Dr. Ann Matthysse, Reader

Dr. Helen Lazear, Reader

Abstract

Staphylococcus aureus (*S. aureus*) is a ubiquitous bacterial infectious agent that is becoming an increasingly problematic threat in hospitals and other patient care environments. In cystic fibrosis patients, abnormally viscous mucus prevents debris from being successfully cleared from the lungs, creating a favorable environment for most *S. aureus* strains. Efforts to build a model of unique lung infection sites are underway, but utilizing such a model requires a robust understanding of *S. aureus* virulence factors. This study explored the variation in virulence factor expression among *S. aureus* clinical isolates. The four main features examined were staphyloxanthin, a carotenoid pigment and chemical virulence factor mucoidy, an indication of *ica* expression; biofilm formation; and accessory gene regulator (*Agr*) function. Carotenoid pigments were extracted and the absorbance values were normalized in comparison to a known sample. The absorbance values of biofilm stained with crystal violet were also recorded. Each isolate was plated on Congo Red agar to determine Mucoid vs. Non-mucoid phenotype. Sheep's blood agar was also used to test the presence of *Agr* protein with each isolate. The samples exhibited a wide variety of pigmentation levels, while the majority of them expressed a non-mucoid phenotype. Comparative analysis revealed that isolates with the mucoid phenotype show a statistically significant (p-value of 0.0414) tendency to produce less carotenoids than the non-mucoid isolates. However, there were no statistically significant correlations between the other virulence factors. Identifying the trends, and lack thereof, in virulence factor expression paved the way to a better understanding of how the combination of these factors impacts the success of *S. aureus* in an infection environment.

Introduction

Staphylococcus aureus (*S. aureus*) is an ubiquitous gram-positive bacteria that lives in a variety of environments, including human microbiota. *S. aureus* also commonly inhabits domesticated animals, from housepets to barnyard livestock [1–5]. As an opportunistic pathogen, *S. aureus* remains passive until activated by conditions favorable for infection initiation. There are numerous pathways *S. aureus* can take to begin an infection, which can progress into a plethora of diseases. To name a few, *S. aureus* can cause pneumonia, endocarditis, osteomyelitis, bacteremia, and sepsis in humans [4]. The form of *S. aureus* infection a person is most likely to contract depends on the opportunities provided to the bacteria.

Certain conditions increase the likelihood of *S. aureus* infections. In hospital settings and nursing homes, the higher concentration of people with weakened immune systems and the use of intravenous therapy provides an advantage for *S. aureus* [6]. The Center for Disease Control reports a decline in bloodstream infections as sanitization and methods of intervening transmission improve [7]. Other communal spaces, such as gyms and correctional facilities, are also conducive to *S. aureus* infection initiation [5,8,9]. The persistence of *S. aureus* in these settings is a cause for concern due to the emergence of antibiotic resistant *S. aureus*. Antibiotic resistance makes *S. aureus* a greater threat, particularly to immunocompromised individuals.

The National Institutes of Health defines immunocompromised as having a weakened immune system and reduced ability to fight infections. A person can become immunocompromised due to a diverse array of circumstances: living with AIDS, cancer, diabetes, malnutrition, immunosuppressive treatments, and certain genetic disorders [10]. Immunocompromised populations are particularly vulnerable to opportunistic pathogens,

including *S. aureus*. Patients with cystic fibrosis form an immunocompromised population that the Conlon Lab specifically aims to help combat *S. aureus* infections.

Cystic fibrosis is a disease caused by loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). The result is a severe lack of respiratory system defenses [11]. *S. aureus* is one of the most prevalent pathogens isolated from cystic fibrosis patient airways [12]. Early *S. aureus* infections are detrimental to cystic fibrosis lungs, paving the way for chronic pneumonia [13]. Unique conditions within cystic fibrosis lungs make the eradication of *S. aureus* challenging. A cystic fibrosis lungs mouse model is currently being developed to facilitate testing of novel treatment plans.

Following the discovery of penicillin, scientists utilized an assortment of antibiotics to combat bacterial infections: erythromycin, gentamicin, streptomycin, vancomycin, etc. [14]. Subsequently, there has been a notable increase of methicillin-resistant *S. aureus* (MRSA) strains and non-growing, dormant 'persister' subpopulations exhibiting high levels of tolerance to antibiotics [15–17]. Antibacterial agents need additional support in the infection environment to successfully eradicate pathogens. Variations within the antagonistic colonies must also be considered to prepare an effective treatment plan.

Deploying the most effective antimicrobial with precision is crucial for successful treatment, but there are numerous variables to consider when designing antimicrobial treatments. For example, Radilinski and Conlon discuss pathogen interaction, microbial interspecies interaction, and metabolic heterogeneity in relation to persisters and cystic fibrosis patients [18]. While previous literature emphasizes the significance of understanding the human microbiome and the interspecies reaction within [19], this study investigated the variable of intraspecies

diversity. Virulence factors are characteristics that contribute to *S. aureus* success in an infection environment. This study analyzed diversity in virulence factor expression amongst clinical isolates from cystic fibrosis patients.

The virulence factors assessed were: pigmentation, mucoidy, biofilm formation, and *agr* protein. Pigmentation encompassed the production of the carotenoid pigment staphyloxanthin. The chemical staphyloxanthin is a virulence factor believed to help *S. aureus* survive assaults from reactive oxygen species [21]. Observations of varying coloration in overnight cultures in tryptic soy broth suggest different levels of carotenoid production between samples. Mucoidy has been linked to mutations in the *ica* locus, which is associated with biofilm formation (12). Isolates with high levels of mucoidy tend to present a wrinkled phenotype on Congo Red agar, while low mucoid levels appear as smooth colonies [12,20]. Biofilm formation refers to *S. aureus* colonies creating a thick membrane that is difficult for antimicrobials to penetrate. This survival advantage was quantified by staining the membrane with crystal violet and measuring absorbance levels. *Agr* locus functionality indicated the activity of a quorum sensing transcriptional unit and encoded a δ hemolysin which formed a band on sheep's blood agar. Quorum sensing allows individual *S. aureus* to estimate the presence of other *S. aureus* as a component of determining if conditions are favorable for infection. Variation amongst individual virulence factor expression was observed before attempting to find correlations between virulence factors.

The goal of this study was to identify expected correlations in the virulence factor expression of *S. aureus* isolates. Specifically, this research evaluated the relationship between carotenoid abundance and mucoidity found in isolated patient samples made available to the Conlon lab for study by UNC Hospital clinics. If a sample presented the mucoid phenotype, it

did not express an abundance of the other known virulence factor indicated by pigmentation. Finding this inverse correlation in virulence factor expression within this set of samples contributed to the Conlon Lab's understanding of infection site environments. Further studies could be used to influence novel treatment designs, especially those tested in a cystic fibrosis mouse lung model.

Methods

Collection and Storage of Samples

Thirty-six *S. aureus* samples were isolated from patients with cystic fibrosis. Sample vials were stored in a -80 °C deep freezer.

Carotenoid Extraction

Carotenoids were extracted from samples in triplicate using methanol and multiple stages of heating and centrifugation, as previously described by Morikawa et. al. [21]. To summarize, 1ml of overnight cultures grown in Tryptic Soy Broth (Remel; Lenexa, KS) were pelleted using a microcentrifuge (Thermo Fisher Scientific; Waltham, Massachusetts) and resuspended in 250 µl methanol. The bacteria were incubated at 55°C for 3 minutes, pelleted at 10,000xg for 3 minutes using the relative centrifugal force setting, and the supernatant containing the extracted carotenoids was removed, and the extraction procedure was repeated once. Triplicates of the common control strain LAC carotenoids served as standard each extraction. The pigmentation of each final product was quantified using a Synergy H1 plate reader (Biotek; Winooski, VT) at a wavelength of 465 nm. To account for variation in pigment production, samples were averaged and standardized against LAC (Sample Identification # 1145).

Mucoid vs. Non-mucoid Characterization

To investigate the expression of mucoid vs. non-mucoid phenotype within the list of isolates, colonies of each isolate were grown on Congo red agar. The Congo red agar plates contained brain heart infusion (BHI) agar (Thermo Fisher Scientific), 3.6% sucrose, 0.5%

glucose, and 0.08% Congo red (Sigma Aldrich; St. Louis, MO) in alignment with the methods of Brooks and Jefferson [12]. The plates were streaked with a portion of the *S. aureus* from each isolated sample, and incubated at 37°C. The plates were examined at 24-hour increments following initial plating to assess colony growth. Identification of the phenotypes would be based on the images from previous work characterizing *S. aureus* samples on Congo red agar [12].

Biofilm Formation

Triplicate overnight cultures of each isolate were diluted 1:200 into fresh TSB. 96-well tissue culture plates (Corning® no. 3599) were inoculated with 100µl of the diluted samples and incubated under static conditions for 24 hours at 37°C. A plate washer (Nunc™) was used to wash the microtiter plates with distilled water. The plates dried for one hour at 65°C. 100µl of 0.4% crystal violet solution was used to stain each well for 5 minutes, followed by a second round of plate washing. The contents of the wells were solubilized with 100µl of 5% acetic acid. A BioTek® Synergy H1 plate reader quantified the absorbance of the adhered, stained cells at a wavelength of 492nm. Isolates with an average absorbance value greater than 0.1 were considered biofilm positive.

Agr Function (Quorum Sensing)

Isolates were grown on sheep blood agar (Tryptic Soy Agar (TSA) with sheep blood, Thermo Fisher Scientific), which shows δ hemolysis when *S. aureus* produces functional Agr protein [24]. RN4220, a laboratory strain known to only produce β hemolysin was inoculated down the center of the sheep blood agar plate. Test isolates were inoculated perpendicular to RN4220 without making direct contact. Inoculated plates were then incubated for 16-18 hours at 37°C,

followed by 1-2 hours at 4°C to promote further hemolysis development. An “arrowhead” indicating δ hemolysis formed between RN4220 and the test isolates positive for *Agr*. The negative control was an *agr*-lacking mutant (HG003 agrC::erm) and its *agr* positive parent strain (HG003) was the positive control. Both controls were grown on each sheep blood agar plate (Thermo Fisher Scientific) with the test isolate triplicates from independent cultures.

Computational Analysis

Logistic regression analysis and Two-Tailed T-Tests in Microsoft Excel were used to quantify correlations between virulence factor data. Multiple regression analysis was performed utilizing an online multinomial logistic model (Statistics Kingdom; Melbourne, Australia).

Results

Carotenoid Expression Quantification

Since staphyloxanthin is the predominant carotenoid pigment in *S. aureus*, quantifying extracted carotenoid pigment levels equates to determining staphyloxanthin abundance. Increased levels of absorption meant higher staphyloxanthin concentration. Only 7 of the 36 samples had an average absorbance greater than 0.1. All of the samples had an average absorbance less than 0.25. There was wide variation in carotenoid expression (Figure 1). To ensure the extraction data were consistent, all samples were compared to the lab strain USA300 LAC (#1145), a well characterized MRSA isolate.

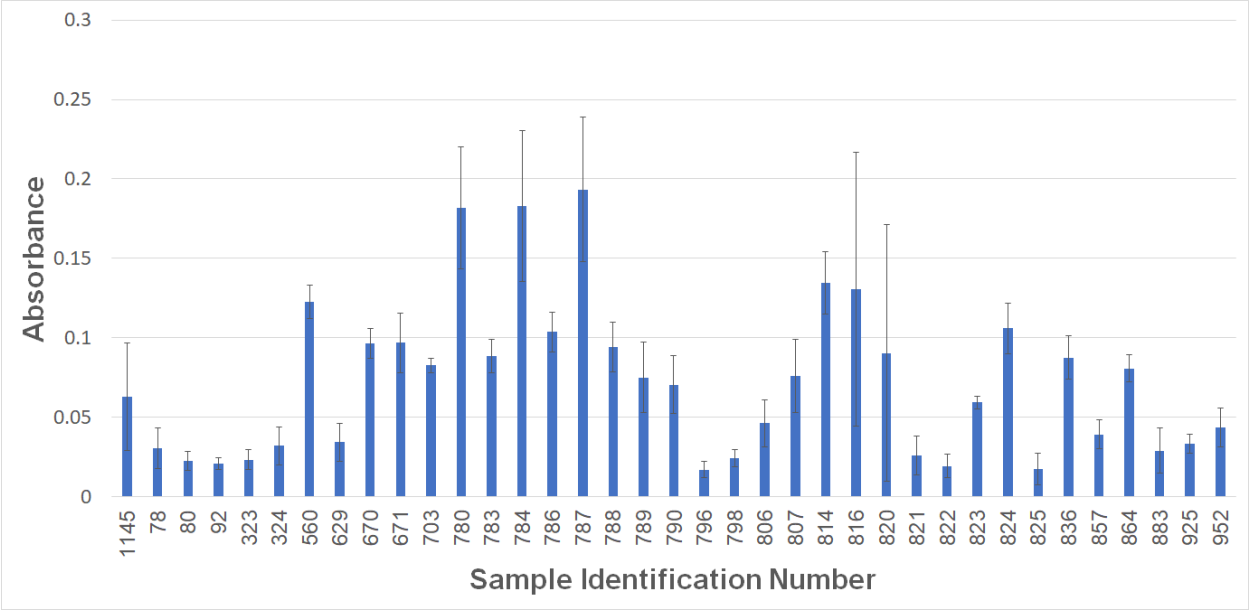

Figure 1.

Pigmentation as indicated by the absorbance levels of each isolate were displayed in comparison to LAC (sample identification # 1145). Error bars represent the standard deviation amongst replicates for each sample. Absorbance was recorded at a wavelength of 465 nm.

Mucoid Phenotype Determination

Colonies of both mucoid (a) and non-mucoid (b, c) phenotype *S. aureus* presented on the plates (Figure 2). Twenty-five of the 36 appeared to be the non-mucoid phenotype. The mucoid phenotype ruffles were more pronounced in certain samples. Occasionally, apparent mucoid phenotype colonies took more than one 24-hour increment to present focal wrinkles. A sample would be considered mucoid, if the colonies appeared to present a wrinkled phenotype in at least two of the four trials conducted.

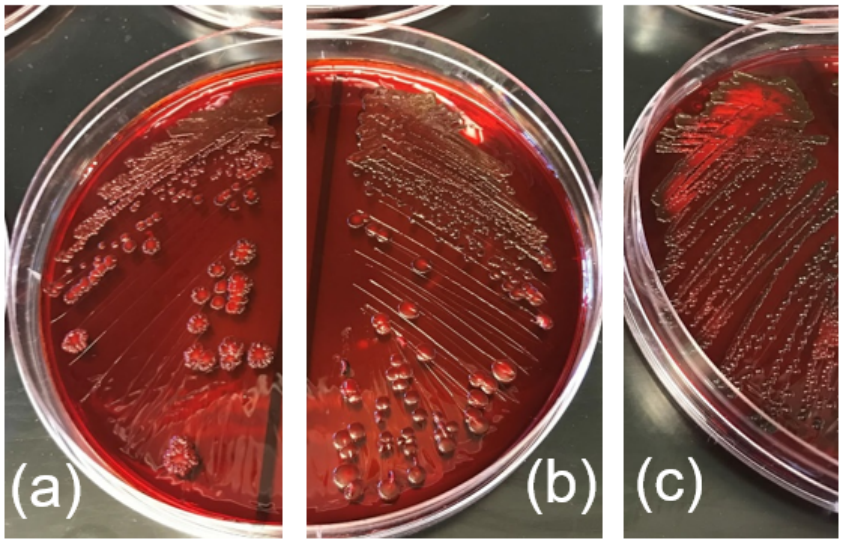

Figure 2. Mucoid vs. Non-mucoid Example

Sample #92 (a) exhibited the wrinkled, mucoid phenotype. Sample #323 (b) was an example of the smooth, non-mucoid phenotype. Sample #324 (c) displayed a darker-hued version of the smooth, non-mucoid phenotype. Photograph taken after ~72 hours of incubation (Date: Feb. 20th 2020).

Biofilm Production Quantification

Biofilm produced by each isolate was stained with crystal violet, which allowed biofilm formation to be quantified by absorbance. Isolates with an average absorbance less than 0.1 were considered biofilm positive. Half of the isolates were recorded as biofilm positive.

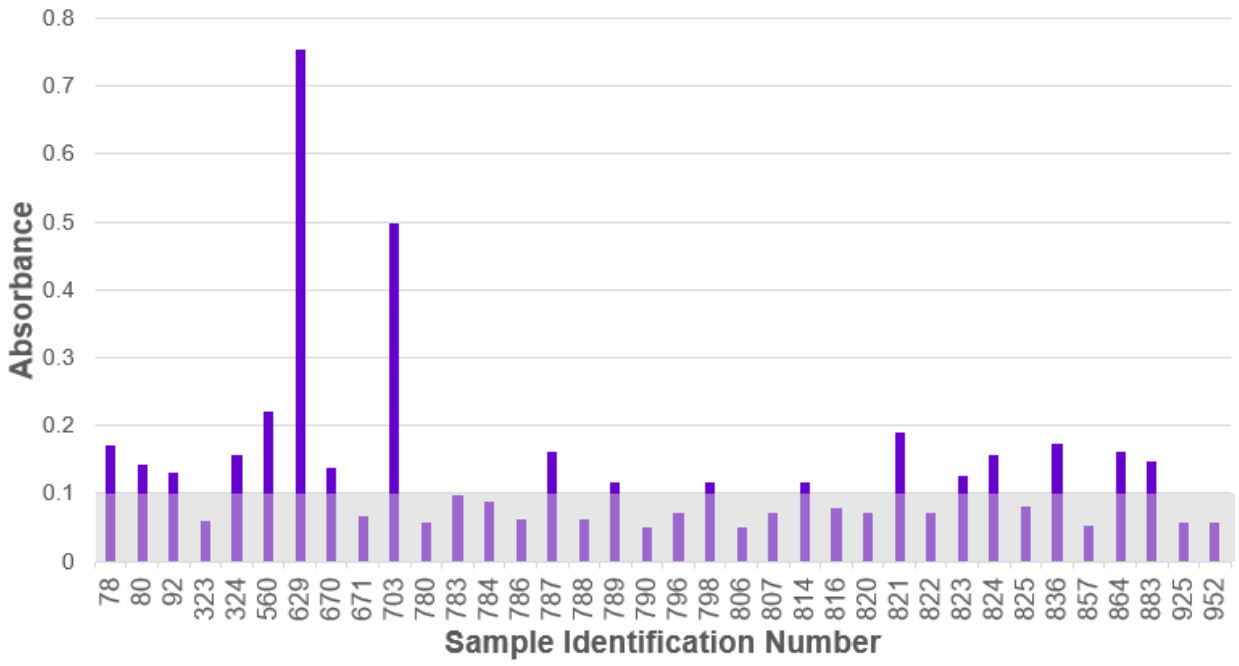

Figure 3.

Biofilm production as indicated by the average absorbance levels of each isolate were displayed in comparison. Absorbance was recorded at a wavelength of 492 nm.

Agr FunctionDetermination

Evaluation of δ hemolysis activity on sheep's blood agar revealed that only 4 of the 36 samples did not have *agr* function. The majority was *agr* (+).

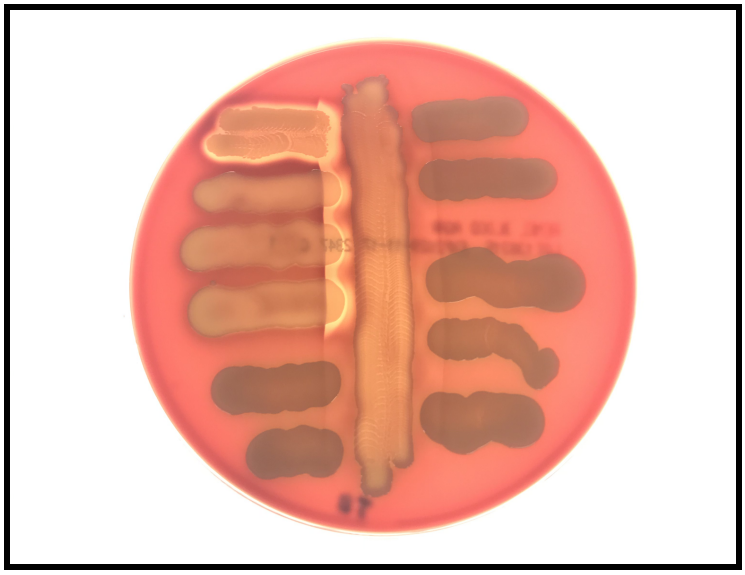

Figure 4.

An example of isolates grown on sheep's blood agar with RN4220 to potentially interact with down the center. In the top right, the large band (almost like two fused together) was the positive control HG003. The top left band was the negative control HG003 agrC::erm. The three bands below HG003 represented triplicates of a positive isolate. The remaining bands consisted of triplicates for two negative isolates.

T-Test Analysis for Significant Correlations

Two sample equal variance T-test were used as one statistical model to identify significant correlations between virulence factors. Every pair combination of the four virulence factors were tested. The p-value cutoff was 0.05. Pigmentation vs. Mucoidy was the only test that produced a statistically significant p-value of 0.0414 (Figure 5). All other combinations did not qualify as statistically significant.

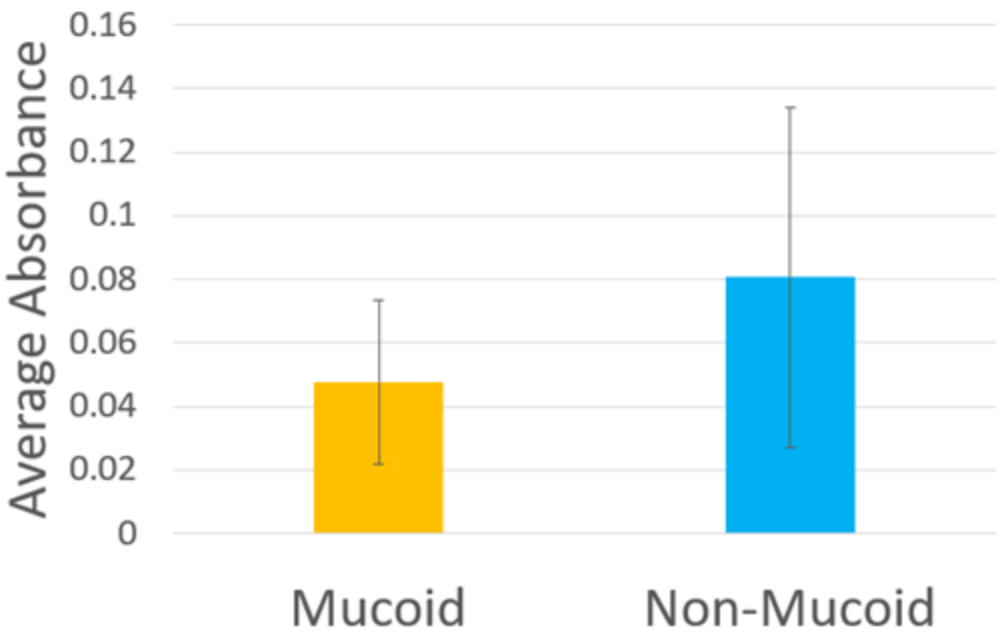

Figure 5.

Averaged absorbance values for mucoid and. non-mucoid samples. Error bars represent standard deviation. A two sample equal variance T-test was performed on the average absorbances of the non-mucoid samples vs. the mucoid samples. The p-value is 0.0414.

Logistic Regression for Continuous vs. Binary Virulence Factors

Basic logistic regression analysis was used as an additional method to quantify the relationship between virulence factors. Logistic regression required one continuous variable to generate predictions regarding a binary variable. Four logistic regressions assessed the influence of the continuous virulence factors (pigmentation and biofilm) on the binary virulence factors (mucoidy and *agr*) (Figure 6). The logistic regressions with the lowest p-value to the greatest are as follows: pigmentation vs. mucoidy ($p = 0.0548$), biofilm vs. mucoidy ($p = 0.140$), pigmentation vs. *agr* ($p = 0.288$) biofilm vs. *agr* ($p = 0.940$). None of the resultant p-values were below p-value cutoff (0.05).

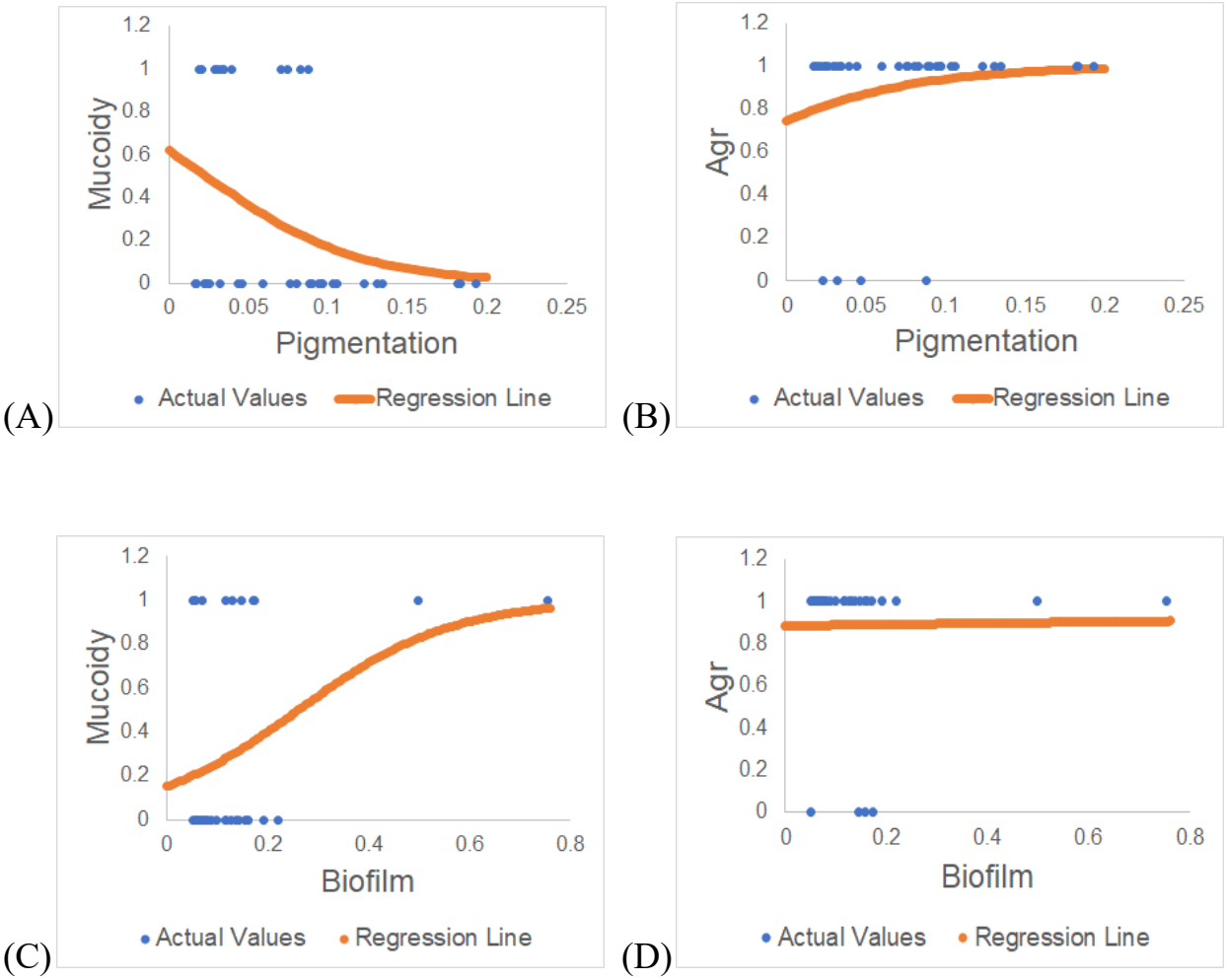

Figure 6.

Actual values represented the true values for each sample along with the calculated logistic regression line for pigmentation absorbance values vs. mucoidy (A; p-value = 0.0548), pigmentation absorbance values and agr protein expression (B; p-value = 0.288), biofilm absorbance data and mucoidy (C; p-value = 0.140), and biofilm absorbance data and agr protein expression (D; p-value = 0.940).

Linear Regression Between Continuous Virulence Factors

To directly compare pigment and mucoidy, a scatter plot was generated (Figure 7). The trendline possessed a low R-squared value of 0.0057. The slope of the line indicates a statistically insignificant negative correlation.

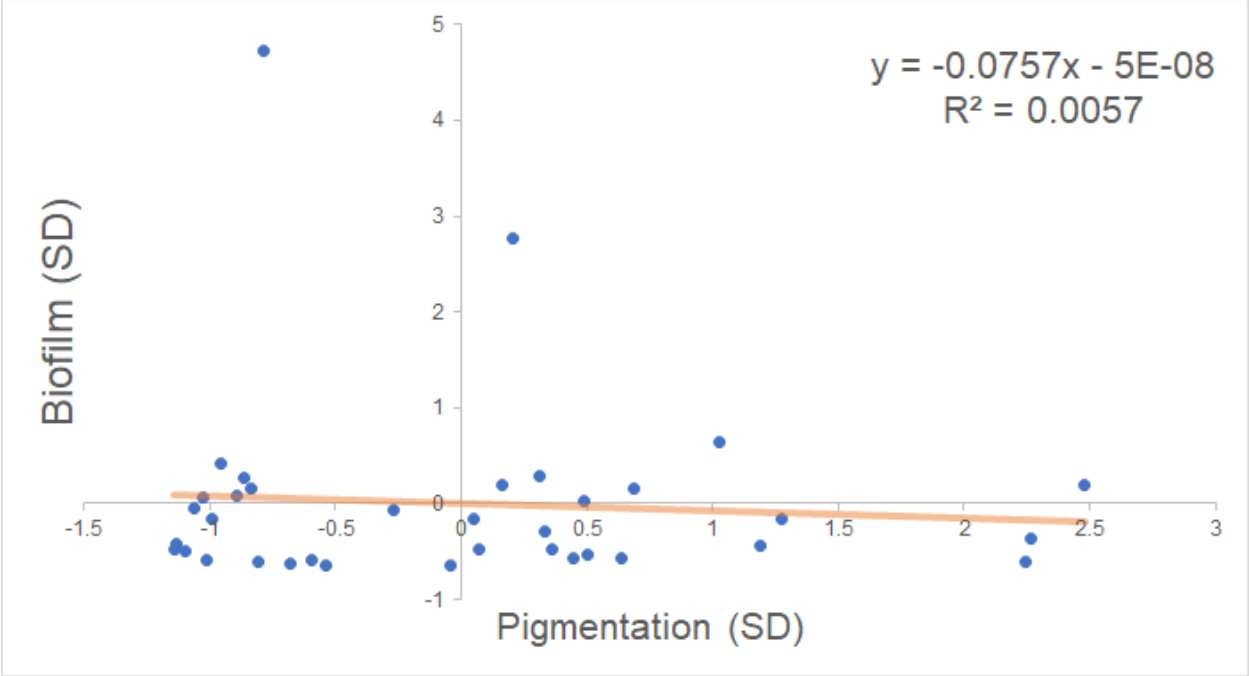

Figure 7

Scatter plot of pigmentation in standard units vs. biofilm in standard units. The trendline and R-squared values are included.

Multiple Logistic Regression Analysis Produced a Four-Variable Model

The combined pigmentation, biofilm, and *Agr* data was used to predict mucoidy in a four-variable model with a favorable distribution (Figure 8 .iii). A significant p-value for pigmentation vs. mucoid was produced (Figure 8 .ii)

(i)	Pigmentation	Biofilm	*Agr*
Pigmentation	1.000	-0.07574	0.1848
Biofilm	-0.07574	1.000	0.01250
Agr	0.1848	0.01250	1.000

(ii)	Coefficient	SE	z-stat	Lower z0.025	Upper z0.975	exp(b)	p-value
b0	1.1279	1.4453	0.7804	-1.7049	3.9608	3.0893	0.4352
Pigment	23.3093	11.8876	1.9608	0.01000	46.6087	>1,000,000	0.04990
Biofilm	-7.8844	5.4375	-1.4500	-18.5417	2.7730	0.0003766	0.1471
Agr	-0.8141	1.2912	-0.6304	-3.3449	1.7167	0.4431	0.5284

(iii)

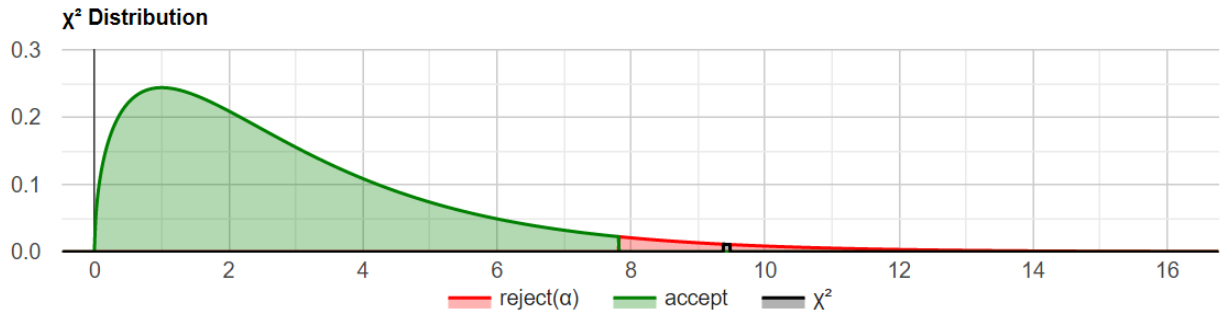

Figure 8

Derived from multiple logistic regression including all four virulence factors: (i) Correlation matrix of the x-values (pigmentation, biofilm, and agr), (ii) table of resulting coefficients, and (iii) X ² distribution.

Discussion

The majority of the statistics indicate that my initial hypothesis that *S. aureus* virulence factors will have significant correlation was not completely accurate. The lack of correlations between the other virulence factors may be influenced by two outliers in the biofilm data (Figure 7). Additionally, more *agr* (-) samples would be beneficial to counter the overwhelming agr (+). My four statistical analysis models all indicated that there is not a significant relationship between the expression of the virulence factors, except for pigmentation with respect to mucoidy.

There appears to be a correlation between pigmentation and mucoidy. All the mucoid phenotype samples had an absorbance of less than 0.1. When the averages of the two types of samples were compared, a significant p-value of 0.0414 was produced (Figure 5). This suggests that the strains with the mucoid phenotype produce less carotenoids than the non-mucoid strains. Conducting more trials with the same samples would be beneficial to verify these observed trends. The process of obtaining the pigmentation and mucoidy results were limited to a few trials each. As observed with the LAC triplicates that accompanied every round of carotenoid extraction, each strain has the potential for high variability in pigment production. Certain colonies appeared to be an intermediate phenotype: smooth yet with the presence of some wrinkles especially around the edge of the colony. The sample needed distinct wrinkles to be considered a mucoid strain and typically developed within the following 24-hour incubation period. Generally, colonies appearing intermediate in feature decreased as incubation time increased.

Previous literature, particularly work from Schwartbeck [12] and Brooks and Jefferson [20] involving Congo red agar plates to test for mucoidy, does not mention any intermediate

between the phenotypes. Brooks and Jefferson report the phenotypic identity of their colonies as straightforward to discern [20]. Perhaps differences in the plating technique or timeline of colony analysis account for the distinction. With additional trials, varying either the plating technique or the timeline of analysis could prove beneficial for the accuracy of *S. aureus* phenotypic identification.

Considering that there is a suggested correlation between the carotenoid production and the mucoidy of *S. aureus* samples, further investigation may reveal patterns involving other characteristics. One particular trait that may be heavily influenced by mucoidy is the formation of biofilm. Studies within The Conlon Lab emphasizes how the prevalence of biofilm in an infection site can complicate the treatment process [22]. The efficacy of the treatments used against *S. aureus* may be improved by understanding the relationship between pigmentation, mucoidy, and pigmentation. Ideally, if the pigmentation or the mucoid phenotype indicate the sample strain's ability to produce biofilm, an appropriate response can be launched to combat that infection. Furthermore, The presence of *Pseudomonas aeruginosa* can affect the characteristics of *S. aureus* in vivo [23]. Perhaps, when with a better understanding of *S. aureus* characteristics and their relationship with *Pseudomonas aeruginosa* we can utilize that information to estimate the abundance of *Pseudomonas aeruginosa* as well. Perhaps, more significant correlations will be revealed as more data is acquired.

References

1. Boost MV, O'Donoghue MM, James A. Prevalence of Staphylococcus aureus carriage among dogs and their owners. Epidemiol Infect. 2008;136: 953–964.

2. Bierowiec K, Płoneczka-Janeczko K, Rypuła K. Is the Colonisation of Staphylococcus aureus in Pets Associated with Their Close Contact with Owners? PLoS One. 2016;11: e0156052.

3. Gilbertie JM, Schnabel LV, Hickok NJ, Jacob ME, Conlon BP, Shapiro IM, et al. Equine or porcine synovial fluid as a novel ex vivo model for the study of bacterial free-floating biofilms that form in human joint infections. PLoS One. 2019;14: e0221012.

4. Public Health Agency of Canada. Pathogen Safety Data Sheets: Infectious Substances – Staphylococcus aureus - Canada.ca. 30 Apr 2012 [cited 14 Apr 2020]. Available: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/staphylococcus-aureus.html

5. McKenna M. Superbug the fatal menace of MRSA. 1st Free Press hardcover ed. New York: Free Press; 2010.

6. Staphylococcus aureus in Healthcare Settings | HAI | CDC. 16 Apr 2019 [cited 14 Apr 2020]. Available: https://www.cdc.gov/hai/organisms/staph.html

7. Kourtis AP, Hatfield K, Baggs J, Mu Y, See I, Epson E, et al. Vital Signs: Epidemiology and Recent Trends in Methicillin-Resistant and in Methicillin-Susceptible Staphylococcus aureus Bloodstream Infections - United States. MMWR Morb Mortal Wkly Rep. 2019;68: 214–219.

8. Maurice Bilung L, Tahar AS, Kira R, Mohd Rozali AA, Apun K. High Occurrence of Staphylococcus aureus Isolated from Fitness Equipment from Selected Gymnasiums. J Environ Public Health. 2018;2018: 4592830.

9. Malcolm B. The rise of methicillin-resistant staphylococcus aureus in U.S. correctional populations. J Correct Health Care. 2011;17: 254–265.

10. Institute NC. NCI dictionary of cancer terms: immunotherapy. Available: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immunocompromised

11. Muraglia KA, Chorghade RS, Kim BR, Tang XX, Shah VS, Grillo AS, et al. Small-molecule ion channels increase host defences in cystic fibrosis airway epithelia. Nature. 2019;567: 405–408.

12. Schwartbeck B, Birtel J, Treffon J, Langhanki L, Mellmann A, Kale D, et al. Dynamic in vivo mutations within the ica operon during persistence of Staphylococcus aureus in the airways of cystic fibrosis patients. PLoS Pathog. 2016;12: e1006024.

13. Li C, Wu Y, Riehle A, Ma J, Kamler M, Gulbins E, et al. Staphylococcus aureus Survives in Cystic Fibrosis Macrophages, Forming a Reservoir for Chronic Pneumonia. Infect Immun. 2017;85. doi:10.1128/IAI.00883-16

14. Tharmalingam N, Rajmuthiah R, Kim W, Fuchs BB, Jeyamani E, Kelso MJ, et al. Antibacterial Properties of Four Novel Hit Compounds from a Methicillin-Resistant Staphylococcus aureus-Caenorhabditis elegans High-Throughput Screen. Microb Drug Resist. 2018;24: 666–674.

15. Kim W, Zhu W, Hendricks GL, Van Tyne D, Steele AD, Keohane CE, et al. A new class of synthetic retinoid antibiotics effective against bacterial persisters. Nature. 2018;556: 103–107.

16. Conlon BP, Nakayasu ES, Fleck LE, LaFleur MD, Isabella VM, Coleman K, et al. Activated ClpP kills persisters and eradicates a chronic biofilm infection. Nature. 2013;503: 365–370.

17. Zalis EA, Nuxoll AS, Manuse S, Clair G, Radlinski LC, Conlon BP, et al. Stochastic Variation in Expression of the Tricarboxylic Acid Cycle Produces Persister Cells. MBio. 2019;10. doi:10.1128/mBio.01930-19

18. Radlinski L, Conlon BP. Antibiotic efficacy in the complex infection environment. Curr Opin Microbiol. 2018;42: 19–24.

19. Chu J, Vila-Farres X, Inoyama D, Ternei M, Cohen LJ, Gordon EA, et al. Discovery of MRSA active antibiotics using primary sequence from the human microbiome. Nat Chem Biol. 2016;12: 1004–1006.

20. Brooks JL, Jefferson KK. Phase variation of poly-N-acetylglucosamine expression in Staphylococcus aureus. PLoS Pathog. 2014;10: e1004292.

21. Morikawa K, Maruyama A, Inose Y, Higashide M, Hayashi H, Ohta T. Overexpression of sigma factor, sigma(B), urges Staphylococcus aureus to thicken the cell wall and to resist beta-lactams. Biochem Biophys Res Commun. 2001;288: 385–389.

22. Radlinski LC, Rowe SE, Brzozowski R, Wilkinson AD, Huang R, Eswara P, et al. Chemical Induction of Aminoglycoside Uptake Overcomes Antibiotic Tolerance and Resistance in Staphylococcus aureus. Cell Chem Biol. 2019;26: 1355–1364.e4.

23. Radlinski L, Rowe SE, Kartchner LB, Maile R, Cairns BA, Vitko NP, et al. Pseudomonas aeruginosa exoproducts determine antibiotic efficacy against Staphylococcus aureus. PLoS Biol. 2017;15: e2003981.

24. Gor, Vishal, et al. “Finding of Agr Phase Variants InStaphylococcus Aureus.” MBio, vol. 10, no. 4, 2019, doi:10.1128/mbio.00796-19.

Appendix

Table 1. List of Samples

Strain Number	Source
BC78	Cystic Fibrosis Clinical Isolate
BC80	Cystic Fibrosis Clinical Isolate
BC92	Cystic Fibrosis Clinical Isolate
BC323	Cystic Fibrosis Clinical Isolate
BC324	Cystic Fibrosis Clinical Isolate
BC560	Cystic Fibrosis Clinical Isolate
BC629	Cystic Fibrosis Clinical Isolate
BC670	Cystic Fibrosis Clinical Isolate
BC671	Cystic Fibrosis Clinical Isolate
BC703	Cystic Fibrosis Clinical Isolate
BC780	Cystic Fibrosis Clinical Isolate
BC783	Cystic Fibrosis Clinical Isolate
BC784	Cystic Fibrosis Clinical Isolate
BC786	Cystic Fibrosis Clinical Isolate
BC787	Cystic Fibrosis Clinical Isolate
BC788	Cystic Fibrosis Clinical Isolate
BC789	Cystic Fibrosis Clinical Isolate
BC790	Cystic Fibrosis Clinical Isolate
BC796	Cystic Fibrosis Clinical Isolate
BC798	Cystic Fibrosis Clinical Isolate

BC806	Cystic Fibrosis Clinical Isolate
BC807	Cystic Fibrosis Clinical Isolate
BC814	Cystic Fibrosis Clinical Isolate
BC816	Cystic Fibrosis Clinical Isolate
BC820	Cystic Fibrosis Clinical Isolate
BC821	Cystic Fibrosis Clinical Isolate
BC822	Cystic Fibrosis Clinical Isolate
BC823	Cystic Fibrosis Clinical Isolate
BC824	Cystic Fibrosis Clinical Isolate
BC825	Cystic Fibrosis Clinical Isolate
BC836	Cystic Fibrosis Clinical Isolate
BC857	Cystic Fibrosis Clinical Isolate
BC864	Cystic Fibrosis Clinical Isolate
BC883	Cystic Fibrosis Clinical Isolate
BC925	Cystic Fibrosis Clinical Isolate
BC952	Cystic Fibrosis Clinical Isolate
BC1145	Lab strain USA 300 LAC

Acknowledgements:

I'd like to express my appreciation to Nikki and the staff of the Conlon Lab for their assistance with many aspects of this project, including teaching me the assay techniques. I would also like to thank the STOR 120 teaching staff for helping me determine my statistical models.

Thank you to the phenomenal faculty UNC faculty who provided me with amazing research experiences. Words cannot express how grateful I am. It truly was my favorite part of my undergraduate experience.